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Agreement between different sleep states and behaviour indicators in dairy cows

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ABSTRACT

Conclusive data regarding behavioural indicators of different sleep states in adult dairy cows are lacking, i.e. agreement between behavioural indicators of sleep and corresponding electrophysiological measures. Behavioural estimates for quantifying total sleep time in calves have been developed, so this study examined whether these behavioural estimates also apply for adult cows.

Behaviour observations and electrophysiological readings were recorded for a total of 13 cows during one recording session per cow lasting on average 4 h 22 min. Recording started when the cow was fully awake and finished when at least one probable sleep bout had been recorded. The behavioural estimates used in the study were: 'lying with head lifted and still' for non-rapid eye movement (NREM) sleep, 'lying with head resting' for rapid eye movement (REM) sleep and 'lying with head lifted and moving' for awakeness. As statistical measures of agreement between behavioural estimates and electrophysiological status (both recorded at 30 s intervals), Cohen's kappa as well as sensitivity and specificity measures were calculated. Additionally, misclassifications were evaluated to better understand agreement between the behaviour and electrophysiological sleep classification. Since interval length might have affected the agreement, the output data were also aggregated into 60, 90 and 120 s intervals and analysed using Wilcoxon sign-rank test to determine the most appropriate interval length.

It was found that the behavioural estimates for assessing total sleep time in calves could not be applied to adult cows as they markedly overestimated NREM and REM sleep time. Behavioural estimates for NREM and REM sleep time were on average 124 ± 17 and 14 ± 4 min per cow, respectively, while the electrophysiological estimate for NREM and REM was on average 20 ± 5 and 10 ± 3 min per cow, respectively. Using the behavioural estimate 'lying with head resting', REM sleep could be identified with moderate precision, but this indicator alone likely underestimates total duration of REM sleep. Behavioural estimates for NREM sleep showed high sensitivity (81%) but low specificity (6%) while the behavioural estimates for REM sleep showed high sensitivity (70%) and moderate specificity (41%). For

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both categories, both sensitivity and specificity increased with increasing measurement interval length. Drowsing as identified from electrophysiological data was present mainly when the cows were lying with head lifted and still, whereas awakeness was present when the cows were lying with head lifted and moving.

1. Introduction

Sleep can be electrophysiologically subdivided into non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. For non-human mammals, the term 'NREM sleep' is often used synonymously with 'slow wave sleep' (Zepelin et al., 2005). In addition to REM and NREM sleep, cows also display an intermediate vigilance state, not well described but commonly referred to as 'drowsing' (Ruckebusch, 1972; Ternman et al., 2012). Drowsiness in humans is a sleep-preparing transitional state experienced when going from awake to asleep and not a lasting state in healthy subjects. In cows, drowsing appears in at least the same proportion as NREM sleep and in the same behavioural posture as for NREM sleep (Ruckebusch, 1972).

Sleep may be electrophysiologically recorded, requiring one recording device per individual. Early studies on sleep in cows used invasive electrophysiological recording, with restrained animals and surgically implanted electrodes (Ruckebusch, 1972). Recently, a non-invasive electrophysiological method for registering sleep was validated in calves (Hänninen et al., 2008) and in adult cows (Ternman et al., 2012). Specific resting postures are reported to be related to the different sleep phases, e.g. during REM sleep phases there is a lack of muscle tone, which is often evident as a relaxed neck (Zepelin et al., 2005). Elephants and giraffes are considered to be in REM sleep when in this posture (Tobler, 1992; Tobler and Schwierin, 1996). However, in these studies no electrophysiological measurements were made to confirm sleep state. The relaxed neck posture has also been suggested to show behavioural sleep in horses and in cows (Ruckebusch, 1972; Williams et al., 2008) and is able to identify 61 ± 7% of REM sleep bouts in calves if the posture is maintained for at least 30 s (Hänninen et al., 2008).

For adult ungulates, no specific NREM sleep posture, other than quiet resting, has been observed. For calves, NREM sleep is often observed when they are lying with their head lifted, but still, for at least 30 s (Hänninen et al., 2008). Sleep postures for elephants and giraffes seem to include standing, but again no electrophysiological recordings have been conducted to validate this (Tobler, 1992; Tobler and Schwierin, 1996). Lack of jaw movements in combination with a recumbent position have been suggested as an indicator of sleep in ruminants (Ruckebusch et al., 1974). This lack of conclusive evidence regarding behavioural indicators of different sleep states demonstrate a need for a more thorough examination of the agreement between behavioural indicators of sleep and corresponding electrophysiological measures in dairy cows. In Ternman et al. (2012) we showed that when the electrophysiological data displayed REM sleep the cow was also observed to be lying with head resting. When electrophysiological data showed drowsing or NREM sleep the cow was observed quietly lying with the head lifted and still. However, the dataset in Ternman et al. (2012) did not allow the reversed comparison to confirm that the body postures seen during REM and NREM sleep always represent sleep or if these body postures are shown in other vigilance states.

Table 1
Behaviour estimates used to describe sleep from observations (Hänninen et al., 2008). NREM = non-rapid eye movement, REM = rapid eye movement.

Electrophysiological state	Behaviour estimate
NREM sleep	Lying with head lifted and still
REM sleep	Lying with head resting against body or object
Total sleep	Lying with head lifted and still, or head resting
Awake	Lying with head lifted and moving

The behavioural estimates for sleep must be validated in order to enable sleep research without electrophysiological measurements. The aim of the present study was to test whether the behavioural estimates found for sleep in calves by Hänninen et al. (2008), specified in Table 1, can be used as measures of sleep in adult cows. Furthermore, scoring interval length has been shown to influence how well sleep states can be confirmed using behavioural estimates (Hänninen et al., 2008) why we also aimed to investigate what interval length might be the most suitable when studying behavioural estimates for sleep in adult cows.

2. Materials and methods

The study was conducted on the research farm at the University of Helsinki, Finland, and at Kungsängen Research Centre, Swedish University of Agricultural Sciences, Sweden. The study was approved by the ethics committee of the University of Helsinki and the Uppsala local ethics committee. Behaviour and electrophysiological data were recorded for a total of 13 cows during one recording session per cow, at a time when no milking or cleaning of the pen was being performed. Recording started when the cow was fully awake and finished when at least one probable sleep bout had been recorded, assuming that sleep was recorded if the cow had shown REM sleep position for more than 2 min. When electrophysiological data were combined with behaviour observation data, recording duration was on average 4 h 22 min (range 1 h 24 min to 5 h 49 min) per cow.

2.1. Animals and management

The 13 cows included in the study were of dairy breeds and comprised five Ayrshires (Finland) and eight Swedish Red Breed (SRB) (Sweden). Lactation number at recording (mean ± SE) was 3.2 ± 0.4 and age was 4.9 ± 0.4 years. Six of the cows (three Ayrshire Breed and three SRB) were lactating and were on average 67 days in milk (DIM) (range 14–177 DIM). The remaining seven cows were dry (two Ayrshires and five SRB), with on average 25 days to estimated calving date (range 114–2 days).

At least 2 h before the recording sessions, the cows were moved from a loose housing system to individual pens (3 m × 3 m) in order to minimise the risk of other cows interfering with the equipment. However, during the recording the cows could always see and hear other cows. The cows had ad libitum access to water through a pressure valve water bowl. Silage and concentrate were fed to meet the individual energy requirements of each cow, following the management routines at each farm. Wood shavings were used as bedding and were replaced daily. Light was manually turned on at 05.30 h in Finland and 07.00 h in Sweden, and turned off at 19.00 h at both locations, with a dim night-light provided. The lactating cows were milked twice daily in the pen, with 10 and 14 h milking intervals.

2.2. Electrophysiological recordings

At a minimum of 1 h prior to the start of the recording process, each cow was fitted with a halter and a harness for holding the recording equipment and was shaved at the electrode attachment sites on the head and neck to ensure sufficient conduction. The patches were then cleaned with alcohol and the electrodes secured with tissue adhesive as described by Ternman et al. (2012). Four electrodes were used for recording brain activity (EEG); one electrode above and one below each eye recorded eye movements with electrooculography (EOG); and two electrodes attached to the neck muscle recorded neck muscle activity (electromyography, EMG) (Fig. 1). The reference electrode was placed in the middle of the forehead and the ground electrode behind the horn base. All electrodes were connected to a mobile recording device (Embla Titanium, Embla Systems Inc., Broomfield, USA) using snap-on cables. The recording device was attached to the harness on the back of the cow. The impedance of the connection was checked before each recording and was in the range 0.2–5.0 kΩ. Data was recorded at 256 Hz and stored on the device until the end of each recording session, and then downloaded to a computer (Ternman et al., 2012).

Fig. 1. Placement of electrodes and equipment used for the electrophysiological sleep recordings (from Ternman et al., 2012).

2.3. Behaviour observations

Cow behaviour was registered by continuous direct observations simultaneously with the electrophysiological recording, by a person positioned next to the pen, visible to the cow. Body and neck postures were scored, along with jaw movements, to identify when rumination occurred. The ethogram is described in detail in Table 2.

2.4. Analysis of electrophysiological data

The EEG, EMG and EOG data were analysed independently from behaviour observations, using digital sleep recording, monitoring and analysing software and visually scored for sleep states (RemLogic 3.2, Embla Systems, Broomfield, USA). The vigilance states NREM sleep, REM sleep, drowsing and awake, were scored visually at 30-s intervals following the Rechtschaffen and Kales (1968) definition for human sleep and the Ternman et al. (2012)

Table 2
Ethogram of scored behaviours by the 13 dairy cows examined in the study.

Behaviour class	Posture	Definition
Body position	Standing	Standing with at least three feet on the ground
	Lying	Lying down on the sternum or side, body to the floor
Head position	Head lifted and moving	Head lifted and moving, supported by the neck
	Head resting	Head resting on the body or ground, not fully supported by the neck
	Head lifted and still	Head lifted and still, supported by the neck
Jaw movements	Rumination	Rhythmic masticatory movements not related to eating
	Other	All other oral behaviours, such as eating, drinking, grooming or no jaw movement

findings on cow sleep. Data from five of the recording sessions used in Ternman et al. (2012) were included, after rescoring of electrophysiological data to ensure congruent scoring of the electrophysiologic data included in this study.

2.5. Statistical analyses

The vigilance states coded from the electrophysiological data in 30-s intervals were connected with the matching behavioural observations. A body and head position as well as the presence or absence of rumination was assigned to each 30 s interval if it lasted for more than half that interval. The statistical analyses were performed using the software R version 2.15.2 (R Core Team, 2013). Data presented are mean ± SE unless otherwise specified.

2.5.1. Agreement between behavioural estimates and vigilance states

Cohen's kappa (κ) values were used to determine the agreement between the electrophysiological and behavioural estimates of vigilance states. κ values of 0.41–0.60 can be regarded as moderate agreement, 0.61–0.80 as substantial agreement and 0.81–1.0 as almost perfect agreement (Landis and Koch, 1977). In addition, sensitivity and specificity (Firk et al., 2002) were calculated to test the predictive value of the behavioural estimates for each vigilance state. Sensitivity (True positive/(True positive + False negative)) describes how well the behavioural sleep estimate can predict occurrence of the actual vigilance state, while specificity (True negative/(True negative + False positive)) describes how well the behavioural sleep estimate can predict non-occurrence of the actual vigilance state. Cross-tabulation between electrophysiological data and the corresponding behavioural sleep estimates was performed to illustratively identify for which vigilance states the possible misclassifications occurred.

2.5.2. Effect of data interval length

In order to test the effect of bout lengths for each vigilance state (data interval lengths), on the accuracy of behavioural estimates, the 30 s data were aggregated into 60, 90 and 120 s intervals. Bouts of vigilance states shorter than the corresponding aggregated interval were excluded, and the behavioural and electrophysiological estimates were compared within cows for differences using a pairwise Wilcoxon signed-rank test.

3. Results

The average time for the observed behaviour 'lying with head lifted and still' was 124 ± 17 min per cow and for 'lying with head resting' 14 ± 4 min per cow. Based on the electrophysiological recordings, the total duration of NREM sleep was 20 ± 5 min per cow, of REM sleep 10 ± 3 min per cow and of drowsing 28 ± 5 min per cow. Sleep as identified from electrophysiological data was only present when the cows were lying down. Drowsing as identified from electrophysiological data was mostly present when the cows were lying in NREM position.

3.1. Agreement between behavioural estimates and vigilance states

The Cohen's κ values for all behavioural estimates showed low to moderate agreement (Fig. 2). The κ values for REM sleep increased with increasing determination interval, while the κ values for NREM sleep, total sleep and drowsing remained very low for all interval lengths. Cohen's κ values for awakeness showed moderate agreement for all interval lengths.

The sensitivity and specificity varied between and within vigilance states (Fig. 3). The sensitivity values for total sleep, NREM sleep and drowsing were high, leading to poor specificity values for these behavioural estimates for all electrophysiological data interval lengths. The behaviour estimates with the highest specificity, along with good sensitivity, were displayed for the vigilance state REM sleep at all interval lengths. The behaviour estimates for awakeness had high specificity and showed moderate sensitivity.

The cross-tabulation showed that the behaviours overlapped for all vigilance states (Fig. 4). However, as shown in Fig. 4, when the behaviours signifying REM sleep were recorded for periods of 60, 90 and 120 s, the vigilance state awakeness was almost never recorded. The best behavioural estimate was found for awakeness, although this might be due to the high number of observations of awakeness (80 ± 12 min per cow) compared with REM

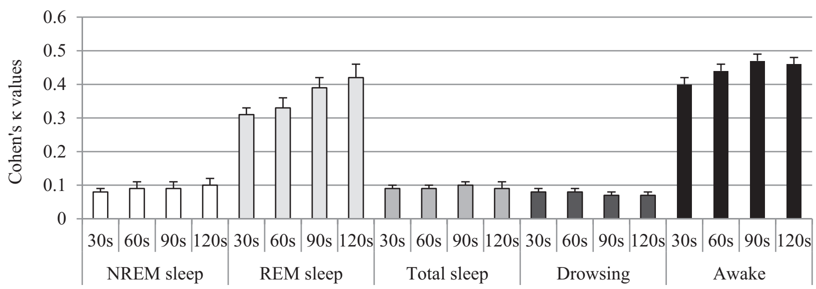

Fig. 2. *Y*-axis shows Cohen's κ-values for behavioural observations and interval length in electrophysiological data. Behaviour combination for the vigilance states NREM sleep and drowsing were lying with head lifted and still, for REM sleep lying with head resting and for total sleep these two behaviour combinations were combined. For the vigilance state awake the behaviour combination was lying with head lifted. Error bars indicate standard error for between-cow variation. NREM = non-rapid eye movement, REM = rapid eye movement.

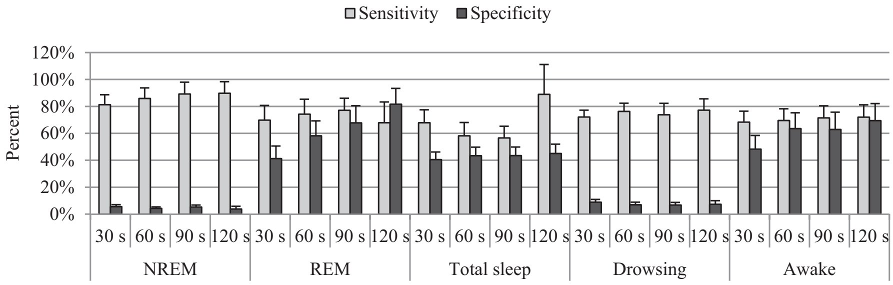

Fig. 3. Sensitivity and specificity values for all electrophysiological and behaviour combinations as well as all interval lengths. The vigilance states NREM sleep and drowsing combined with the behaviour 'lying with head lifted and still', REM sleep combined with 'lying with head resting' and awakeness combined with 'lying or standing with head lifted and moving'. Error bars indicate standard error for between-cow variation. NREM = non-rapid eye movement, REM = rapid eye movement.

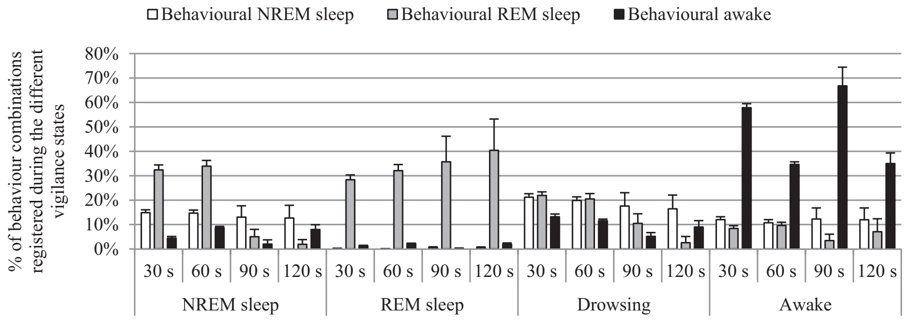

Fig. 4. Average percentage (number of intervals) of behaviour combinations registered during the different electrophysiologically recorded vigilance states for all interval lengths 30, 60, 90 and 120 s. Behavioural NREM refer to the behaviour combination lying with head lifted and still, behavioural REM sleep refer to lying with head resting and behavioural awake refer to lying with head lifted and moving. Error bars indicate standard error for between-cow variation. NREM = non-rapid eye movement sleep, REM = rapid eye movement.

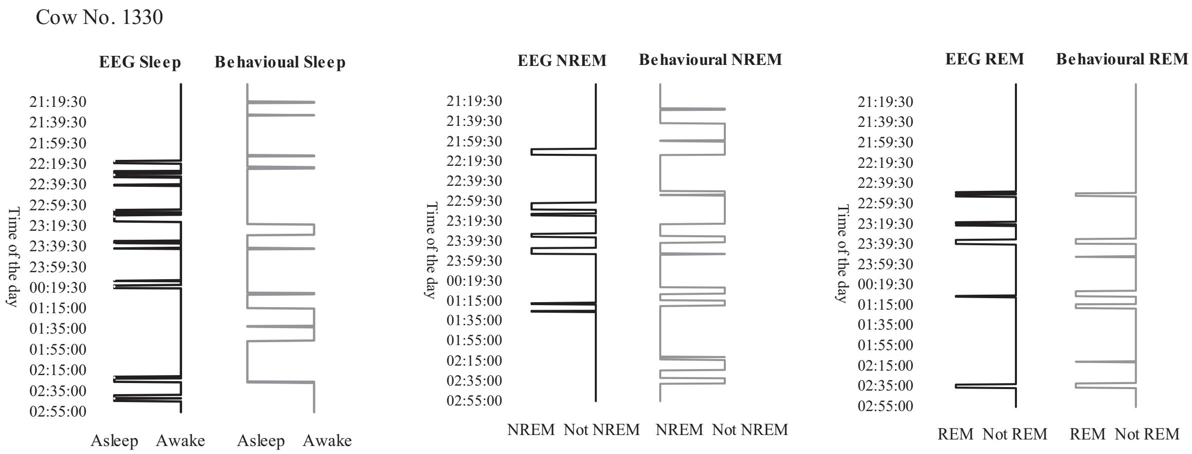

Fig. 5. Sleep pattern during a 5.5-h period (time 21:19–02:55) shown as a comparison between electrophysiological sleep and behavioural sleep. EEG = encephalogram used in electrophysiological recordings, NREM = non-rapid eye movement, REM = rapid eye movement.

sleep (10 ± 3 min per cow), NREM sleep (20 ± 5 min per cow) and drowsing (28 ± 5 min per cow). As shown in Fig. 4, both drowsing and NREM sleep were to a large extent exhibited during the behavioural indicator for NREM sleep.

A descriptive comparison of electrophysiologically recorded sleep pattern and behavioural sleep pattern during one recording session showed that both total sleep time and NREM sleep time were highly overestimated when only the behavioural estimates were used (Fig. 5). During the recording shown in Fig. 5, the REM sleep bouts were fewer than the NREM sleep bouts. Furthermore, the bouts of behavioural REM sleep and electrophysiologically recorded REM sleep were more consistent (Fig. 5).

The median duration for behavioural REM sleep and median duration for electrophysiologically recorded REM sleep did not differ (Table 3). However, with increasing interval length, short REM sleep bouts were removed from the data and for the 120 s intervals only nine cows displayed REM sleep, whereas 10 cows displayed REM sleep in the electrophysiological data when 30 s intervals were used

Table 3
Median duration given in absolute values (min) of behavioural and electrophysiological estimates of sleep for the different data interval lengths. Figures in brackets indicate the interquartile range (IQR). *P*-values refer to Wilcoxon test and *N* = number of cows, NREM = non-rapid eye movement, REM = rapid eye movement.

Vigilance state	Median (interquartile) measures	Interval length			
		30 s	60 s	90 s	120 s
NREM sleep (min)	Electrophysiological estimate	14.00 (36.8)	12.00 (25.5)	12.00 (23.25)	12.00 (20.00)
	N	11	11	10	9
	Behavioural estimate	137.00 (88.00)	104 (74.00)	108.00 (68.25)	100.00 (70.00)
	N	13	13	13	13
	Median difference	106.50 (69.75)	92.00 (67.50)	94.50 (60.85)	88.00 (59.00)
	P	0.01	0.01	0.01	0.01
REM sleep (min)	Electrophysiological estimate	5.00 (15.50)	5.00 (11.50)	4.50 (9.00)	4.00 (7.00)
	N	10	10	10	9
	Behavioural estimate	10.50 (18.25)	7.00 (11.50)	7.50 (11.25)	4.00 (7.00)
	N	12	11	9	8
	Median difference	3.50 (8.50)	1.00 (6.00)	1.50 (6.75)	0.00 (5.00)
	P	0.07	0.23	0.30	0.67
Total sleep (min)	Electrophysiological estimate	37.00 (112.00)	17.00 (38.00)	10.00 (20.00)	7.00 (13.00)
	N	12	12	11	10
	Behavioural estimate	141.00 (103.00)	119.00 (81.50)	112.50 (72.75)	100.00 (72.00)
	N	13	13	13	13
	Median difference	115.00 (71.75)	95.00 (64.50)	91.50 (66.00)	86.00 (63.00)
	P	0.01	0.01	0.01	0.01

(Table 3). Both NREM sleep and total sleep differed considerably between the electrophysiological and observed behavioural estimates, with median values of 12 min and 17 min per cow, respectively, recorded electrophysiologically and 108 and 119 min per cow, respectively, recorded as behaviours.

4. Discussion

This study showed that the behavioural sleep estimates described for calves (Hänninen et al., 2008) cannot be used to estimate sleep in adult dairy cows. The sensitivity of the behavioural estimates for sleep in calves is moderate (54% for NREM sleep), but the specificity of the behavioural estimate for NREM sleep is good (89%) (Hänninen et al., 2008). In contrast, for adult cows the behavioural estimate of NREM sleep displayed high sensitivity (81% for the 30 s interval length) but low specificity (6% for the 30 s interval length). The low specificity was due to our behavioural definition of sleep as drowsing and NREM sleep are characterised by the same behaviour. The vigilance state drowsing is not displayed in calves (Hänninen et al., 2008), which could explain why the behavioural sleep estimates were more accurate in estimating total sleep time for calves. Furthermore, we found that NREM and REM sleep could not be separated in cows using the behavioural estimates of sleep.

The behavioural estimates of sleep overestimated total sleep time in cows, mean behavioural NREM and REM sleep time was 124 ± 17 and 14 ± 4 min per cow respectively, compared with the mean electrophysiologically recorded NREM and REM sleep time of 20 ± 5 and 10 ± 3 min per cow respectively. Only one-third of the behavioural estimates for NREM sleep were actually confirmed by the electrophysiological data. According to Ruckebusch (1972), the majority of total sleep time consists of NREM sleep, which probably is the reason for the total sleep time overestimation using behaviour indicators in the present study.

The results of the present study are in line with findings by Ruckebusch (1972) that cows can both drowse and be awake in a lying position, undermining the possibility to conduct sleep studies using behaviour measures only. The different behavioural estimates for NREM sleep, REM sleep and awakeness were all observed during the vigilance state drowsing. Drowsing has been suggested to be an intermediate state between awake and sleep in cows (Ruckebusch, 1972), but its function is not well known. However, according to our previous electrophysiological findings, drowsing in dairy cows might be regarded as light sleep (Ternman et al., 2012) but further studies are needed to define drowsing in dairy cows.

The behavioural estimate for awakeness was able to distinguish the vigilance state awake accurately. However, during some of the registrations for behavioural NREM or REM sleep estimates, awakeness was recorded electrophysiologically. This was most likely due to that when the cows woke up from NREM and REM sleep they stayed in a sleeping position, as described previously for calves (Hänninen et al., 2008). Hänninen et al. (2008) showed that if calves were observed in a sleep position for more than 30 s they were most likely asleep, although this was not seen for the adult cows.

As cows are less active than calves, we tested whether increasing the output data interval length would improve the behavioural estimates. Increasing the interval length from 30 s to 120 s strengthened the behavioural estimate for REM sleep and awakeness in cows. However, Ternman et al. (2012) showed that the average REM sleep bout duration for adult cows is 3 ± 2 min and increasing interval length may therefore reduce the total estimated REM sleep time. In the present study, REM sleep was lost for one cow when the interval length was increased. Thus, the behavioural estimate for REM sleep could be used to identify episodes of REM sleep, although it would not be possible to quantify the REM sleep using the behavioural estimates alone.

5. Conclusions

Behavioural estimates for assessing total sleep time in calves could not be used to estimate total sleep time in adult cows. The behavioural estimate 'lying with head resting' allowed REM sleep in adult cows to be identified with good precision and increasing interval length improved the estimates. However, behavioural estimates alone are not sufficient to determine and quantify REM sleep time, since short bouts of REM sleep may be overlooked.

Conflict of interest

There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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